

What is claimed is:

1. An isolated nucleic acid molecule encoding a polypeptide which has 2,5-DKG permease activity.
2. The isolated nucleic acid molecule of claim 1,
5 comprising a nucleotide sequence having at least 40% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.
3. The isolated nucleic acid molecule of claim 1,
10 comprising a nucleotide sequence having at least 80% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.
4. The isolated nucleic acid molecule of claim 1,
comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9 and 11.
- 15 5. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence which encodes a polypeptide having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.
- 20 6. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence which encodes a polypeptide having at least 80% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

7. The isolated nucleic acid molecule of claim 1, which encodes a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8, 10 and 12.

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8. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

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9. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of at least any two of SEQ ID NOS:4, 8 and 12.

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10. The isolated nucleic acid molecule of claim 1, which comprises a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of at least any two of SEQ ID NOS:2, 6 and 10.

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11. The isolated nucleic acid molecule of claim 1 operatively linked to a promoter of gene expression.

12. The isolated nucleic acid molecule of claim 11, wherein said promoter is a *lac* promoter.

13. A vector comprising the isolated nucleic acid molecule of claim 11.

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14. The vector of claim 13, comprising a spectinomycin resistance gene.

15. A bacterial cell, comprising the vector of claim 13.

16. The bacterial cell of claim 15, wherein said isolated nucleic acid molecule comprises a nucleotide
5 sequence which encodes a polypeptide having an amino acid sequence at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10 and 12.

17. The bacterial cell of claim 16, wherein said
10 amino acid sequence is at least 95% identical to SEQ ID NO:8.

18. The bacterial cell of claim 17, further comprising an isolated nucleic acid molecule comprising a nucleotide sequence which encodes a polypeptide having an
15 amino acid sequence at least 95% identical to SEQ ID NO:4.

19. The bacterial cell of claim 17, further comprising an isolated nucleic acid molecule comprising a nucleotide sequence which encodes a polypeptide having an amino acid sequence at least 95% identical to SEQ ID NO:10.

20. The bacterial cell of claim 15, which is of the genus *Klebsiella*.

21. The bacterial cell of claim 15, which is deficient in endogenous 2,5-DKG activity.

22. The bacterial cell of claim 21, comprising an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:14 and 2-keto reductase activity.

5 23. The bacterial cell of claim 21, comprising an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:16 and 5-keto reductase activity.

24. The bacterial cell of claim 15, which is of
10 the genus *Pantoea*.

25. The bacterial cell of claim 15, which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-KLG.

26. The bacterial cell of claim 25, which
15 expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

27. The bacterial cell of claim 26, which is deficient in endogenase 2-keto-reductase activity.

28. An isolated oligonucleotide, comprising at
20 least 20 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7 and 9.

29. The isolated oligonucleotide of claim 28, comprising at least 50 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7 and 9.

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30. An isolated oligonucleotide, comprising a nucleotide sequence encoding a peptide having at least 10 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8 and 10.

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31. A method of making the isolated nucleic acid molecule of claim 1, comprising introducing into a bacterial cell deficient in endogenous 2,5-DKG permease activity one or more expressible nucleic acid molecules, identifying a cell having 2,5-DKG permease activity following said
15 introduction, and isolating the introduced nucleic acid molecule from said cell.

32. The method of claim 31, wherein said one or more isolated nucleic acid molecules is a genomic DNA library.

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33. The method of claim 32, wherein said genomic DNA library is prepared from an environmental sample.

34. The method of claim 31, wherein said bacterial cell is a *Klebsiella oxytoca* deficient in *yiaX2*.

35. The method of claim 31, wherein said bacterial cell comprises an isolated nucleic acid molecule encoding a polypeptide having at least 80% identity to SEQ ID NO:14 and 2-keto reductase activity, and a polypeptide
5 having at least 80% identity to SEQ ID NO:16 and 5-keto reductase activity.

36. A method of using the isolated nucleic acid molecule of claim 1 to enhance 2-KLG production, comprising expressing the polypeptide encoded by said nucleic acid
10 molecule in a bacterial which expresses an enzyme that catalyzes the conversion of 2,5-DKG to 2-KLG.

37. The method of claim 36, wherein said bacterial cell further expresses enzymes that catalyze the conversion of glucose to 2,5-DKG.

15 38. The method of claim 37, wherein said bacterial cell is deficient in endogenous 2-keto reductase activity.

39. The method of claim 36, wherein said
20 bacterial cell is of the genus *Pantoea*.

40. The method of claim 36, further comprising converting said 2-KLG to ascorbic acid.

41. An isolated polypeptide which has 2,5-DKG permease activity.

42. The isolated polypeptide of claim 41, comprising an amino acid sequence having at least 40% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

5 43. The isolated polypeptide of claim 41, comprising an amino acid sequence having at least 80% identity to an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

10 44. The isolated polypeptide of claim 41, comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

45. The isolated polypeptide of claim 41, comprising at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8, 10 and 12.

15 46. An isolated peptide, comprising at least 10 contiguous amino acids of any of SEQ ID NOS:2, 4, 6, 8 and 10, wherein said peptide is immunogenic.

47. An antibody specific for the isolated polypeptide of claim 44.

20 48. An antibody specific for the isolated peptide of claim 46.